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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant:	Xinnian Dong et al.	Art Unit:	1649
Serial No.:	08/908,884	Examiner:	A. Nelson
Filed:	August 8, 1997		
Title:	ACQUIRED RESISTANCE GENES AND USES THEREOF		

Assistant Commissioner of Patents
Washington, D.C. 20231

DECLARATION OF XINNIAN DONG, PH.D.

I declare:

1. I am a co-inventor of the subject matter described and claimed in the above-captioned patent application.

2. As disclosed in the above-referenced patent application, we have cloned the *NPR1* gene, a gene that encodes a protein with ankyrin repeats. Overexpression of *NPR1* has been found to confer, in the dicot *Arabidopsis*, enhanced disease resistance to a broad spectrum of plant pathogens, including bacterial and oomycete pathogens.

3. In collaboration with Dr. Pamela C. Ronald of the University of California, Davis, we also have shown that the *Arabidopsis NPR1*-mediated defense pathway functions in the monocot rice. In particular, following the teaching of the above-

referenced application and standard methods known when the application was filed, we overexpressed the *Arabidopsis NPR1* gene in rice and challenged the rice plants with *Xanthomonas oryzae* pv. *oryzae* (*Xoo*), the causal agent of rice blight.

4. To overexpress *NPR1* in rice, we constructed *Ubi-NPR1* and *35S-NPR1* plasmids in which expression of the *Arabidopsis NPR1* cDNA was driven by either a maize ubiquitin (*Ubi*) promoter or the CaMV *35S* promoter, respectively. *Agrobacterium*-mediated transformation of the rice cultivar Taipei (TP) 309 was then used to generate transgenic plants carrying these two constructs. After regeneration, six-to-eight week old rice plants were challenged with *Xoo* Philippine race 6 to test for enhanced resistance using standard pathogen inoculation experiments. We tested at least twenty *Ubi-NPR1* and twenty-nine *35S-NPR1* independent transgenic lines for disease resistance. Genomic DNA blot hybridization and polymerase-chain-reaction (PCR) analyses confirmed the presence of the *NPR1* transgene in the transgenic rice lines.

5. In these inoculation experiments, the transformation recipient TP309 and the IRBB21 cultivar or an *Xa21*-transgenic line (Song et al., *Science* 270:1804-1806, 1995) were included for comparison. Both IRBB21 and *Xa21* plants contain the *Xa21* gene and are resistant to *Xoo* Philippine race 6. TP309 plants are susceptible to *Xoo* Philippine race 6 and developed on average a leaf lesion length of nine cm or longer, whereas IRBB21 and *Xa21* plants yielded lesion lengths of two cm or shorter. Many of the *Ubi-NPR1* lines developed lesion lengths significantly shorter than that of TP309,

indicating that overexpression of *NPR1* in rice leads to enhanced resistance to bacterial blight. In general, the *Ubi-NPR1* lines showed higher resistance than the *35S-NPR1* lines.

6. Further analysis of disease resistance was conducted using the *Ubi-NPR1* lines. T1 plants of several independent lines were tested for segregation of the enhanced resistance to *Xoo* Philippine race 6. The *NPR1*-enhanced resistance phenotype was found to segregate among the T1 plants tested. In inoculation experiments, T1 *Ubi-NPR1* plants exhibited reduced lesion lengths compared to TP309, confirming the inheritance of the enhanced resistance phenotype. The resistance resulting from *NPR1* overexpression was less effective when compared to rice plants carrying the *Xa21* gene, as indicated by the shorter leaf lesion lengths of *Xa21* plants.

7. RNA blot hybridization was utilized to analyze the correlation between *NPR1* expression levels and the enhanced resistance phenotype. RNA blot hybridization results of several segregating T1 plants showed that enhanced disease resistance to *Xoo* was correlated with the presence of *NPR1* mRNA. Further analysis of these hybridization results demonstrated that the enhanced resistance phenotype was tightly correlated with high levels of steady-state *NPR1* mRNA in the transgenic rice plants.

8. In conclusion, since transgenic rice plants overexpressing the *Arabidopsis NPR1* gene displayed enhanced resistance, it is not unreasonable to conclude that rice shares with *Arabidopsis* a similar disease resistance pathway mediated by *NPR1*.

Moreover, I note that these results substantiate the teachings found throughout the specification of the above-referenced application; namely that genes, like the *Arabidopsis NPR1* gene, exist in other plants such as rice, and encode resistance proteins functionally equivalent to the *Arabidopsis NPR1* protein.

9. All statements made herein of my knowledge are true and all statements made on information and belief are believed to be true; and further these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment or bother under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Date 2/7/01

Xinnian Dong, Ph.D.

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